

Alcohol increases homocysteine and reduces B vitamin concentration in healthy male volunteers—a randomized, crossover intervention study

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Summary

Background: Few studies have examined the effect of alcohol consumption on total homocysteine (tHcy) concentrations.

Aim: To assess the effect of an 8-week intervention with vodka or red wine on plasma tHcy and B vitamin concentrations in healthy male volunteers. To assess the effect on tHcy according to methylenetetrahydrofolate reductase (MTHFR) 677C>T genotype.

Design and methods: A randomized controlled crossover intervention study measuring tHcy and serum folate and vitamin B₁₂ concentrations was conducted in 78 male subjects (21–70 years). Following a 2-week washout period during which no alcohol was consumed, all subjects consumed 24 g alcohol (either 240 ml red wine or 80 ml vodka)/day for a 2-week period. Following a further 2-week washout, participants consumed the alternate intervention for 2 weeks.

Results: A significant increase in plasma tHcy was observed after the 2-week red wine intervention (5%, $P=0.03$), and a non-significant increase in tHcy with vodka intervention (3%, $P=0.09$). When the two interventions were compared, the change in tHcy did not differ between the vodka and red wine interventions ($P=0.57$). There were significant decreases in serum vitamin B₁₂ and folate concentrations, and this decrease did not differ between interventions. The increase in tHcy observed in both interventions did not vary by MTHFR 677C>T genotype.

Conclusions: A 2-week alcohol intervention resulted in a decrease in folate and vitamin B₁₂ status and an increase in plasma tHcy. The effect of alcohol intervention on tHcy, folate and vitamin B₁₂ concentrations did not differ between the red wine and vodka intervention groups.

Introduction

Human beings have for centuries consumed alcoholic beverages.¹ Current opinion, based on epidemiological studies, suggests that a low or moderate consumption of alcohol has a protective effect on cardiovascular disease (CVD).^{2,3} The relationship

between alcohol consumption and cardiovascular mortality is a J-shaped curve,⁴ with the lowest mortality at an alcohol consumption of 2–4 U (16–32 g) of alcohol per day.^{4,5} At higher levels of alcohol intake, it has been suggested that the

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individual will encounter various physiological, psychological and pathological changes detrimental to good health.^{2,6-8}

The cardioprotective effect of moderate alcohol intake has not yet been clearly defined, although it is known that beneficial changes in serum lipids are produced.^{4,9} Moreover, it is still not clear whether one type of alcoholic drink is more protective than another,¹⁰⁻¹² although it has been suggested that antioxidants in red wine may lead to additional health-promoting effects beyond those attributable to its alcohol content.¹³

Total homocysteine (tHcy) is recognized as a CVD risk factor.^{14,15} It is elevated in patients with chronic alcoholism and falls following alcohol withdrawal;¹⁶ therefore, alcohol may have a deleterious effect on health by raising tHcy levels. Homocysteine is regulated through a series of pathways, which are dependent on B vitamins, particularly folate.^{14,17} A common genetic variant of the methylenetetrahydrofolate reductase (MTHFR) 677C>T gene has been associated with an increase in tHcy levels, particularly in those who have low folate status.¹⁸ A number of observational studies have suggested an association between moderate alcohol consumption and tHcy concentrations,^{19,20} and an association between alcohol, folate and chronic disease risk has been observed.²¹ However, little randomized trial evidence is available for examining the effect of alcohol on tHcy, and, in particular, it remains to be conclusively determined whether the effect of alcohol on tHcy concentrations depends on the type of alcoholic beverage. In addition, it is not clear whether the MTHFR 677C>T genotype affects response to alcohol intervention.

The aims of this study were 2-fold:

- To assess the effects of a 2-week intervention with red wine or vodka on tHcy, folate and vitamin B₁₂ in healthy male participants.
- To assess whether the effects of the intervention differ according to MTHFR 677C>T genotype.

Methods

Subjects

This was a randomized, crossover intervention study and was approved by the Ethics Committee of Queen's University Belfast. A total of 85 healthy male participants aged between 21 and 70 years were recruited from hospital staff and members of the public. Those consuming >21 U alcohol/week, those with abnormal liver profiles, those taking vitamin supplements and those with any serious concurrent illness were excluded.

Following an initial 2-week run-in period when no alcohol was consumed, subjects drank either 240 ml of red wine (Jacob's Creek Shiraz Cabemet, 1994, South Eastern Australia) or 80 ml of vodka (Smirnoff vodka) for a 2-week period. This is equivalent to 24 g of ethanol per day (corresponding to 3 U of alcohol) and represents an intake that is associated with low overall mortality,²² and is in keeping with current UK guidelines on safe levels of alcohol consumption. Following a further 2-week alcohol free washout period, subjects consumed the other alcoholic drink for a further 2 weeks. No other alcohol was consumed during the course of the study. A full clinical assessment, including assessment of height, weight and blood pressure was carried out at the beginning of the study, and subjects were asked not to change their physical activity or dietary habits during the course of the study. Advice was given regarding suitable mixers for the vodka intervention (i.e. could not be fruit juice).

Blood sampling

Fasting venous blood samples were collected at the beginning and end of each 2-week period. A blood sample anticoagulated with EDTA was stored on ice, whilst a further sample was left to clot for 45 min after venepuncture. Serum and plasma were separated by centrifugation at 3000 rpm for 10 min at 4°C and stored at -70°C until analysis.

Laboratory methods

All chemicals were purchased from Sigma Chemical Company, Poole, Dorset, UK, unless otherwise stated.

tHcy (i.e. free plus protein bound) was assayed by high-performance liquid chromatography with fluorescence detection according to Ubbink *et al.*²³ Concentrations of serum vitamin B₁₂ and folate were measured by radioassay using a Simultrac kit from ICN Pharmaceuticals, California, USA.

Serum total cholesterol was estimated using an enzymatic CHOD-PAP kit, while serum triglycerides were measured using the Peridochrom GPO-PAP kit (both Boehringer Mannheim, Mannheim, Germany). Precipitation for HDL-cholesterol estimation employed phosphotungstic Mg²⁺ reagents. All cholesterol assays were carried out on a Cobas Fara auto-analyser.

DNA extraction and genotyping

Genotyping for the MTHFR 677C>T polymorphism was performed by PCR and *Hinf*I digestion as in Frosst *et al.*²⁴

Statistical analysis

Subjects were only included in the final analysis if they completed the full study protocol, i.e. analysis was carried out on a per protocol basis. Analysis of this two-period crossover study was carried out according to Hills and Armitage.²⁵ There were no significant period or carry-over effects, and therefore paired samples *t*-tests were used to compare the intervention groups. All analyses were carried out using STATA software (version 9.0, StataCorp, TX, USA) and SPSS (version 11).

Results

The baseline characteristics of the study participants, according to intervention group, are shown in Table 1.

A total of 85 participants were recruited into the study, and 78 participants completed the full study protocol. Table 2 shows the ratio of geometric means (post-intervention : pre-intervention), or the difference in means (post-intervention – pre-intervention) in each intervention group. A significant increase in plasma tHcy was observed after the 2-week red wine intervention [ratio of geometric means (95% CI) 1.05 (1.01–1.09), *P*=0.03], with a non-significant increase in tHcy with vodka intervention [1.03 (0.99–1.07), *P*=0.09]. When the two interventions were compared, the change in tHcy did not differ between the vodka and red wine interventions (*P*=0.57). There were significant decreases in serum vitamin B₁₂ and folate concentrations during both interventions, and this decrease did not differ between them (*P*=0.94, 0.38, respectively). Concentrations of triglycerides, total cholesterol and HDL-cholesterol increased significantly with the vodka intervention, and these changes were also either significant (total cholesterol) or approached significance (triglycerides, HDL cholesterol) with the red wine intervention. There were no significant differences in the change in lipids between the vodka and red wine interventions.

The change in tHcy by the MTHFR 677C>T genotype is shown in Table 3. The increase in tHcy did not differ between these genotypes, although there was a single outlier (the same participant) in each intervention phase, a MTHFR 677TT homozygote, who had a large increase in tHcy (>20 µmol/l) in each intervention phase. When this outlier was removed the estimates did not change markedly. There was no difference in the effects of the alcohol intervention on folate or vitamin B₁₂

Table 1 Baseline characteristics of study participants by intervention group during first 2-week intervention period

	Intervention group (during first intervention phase)	
	Vodka	Red wine
<i>n</i>	45	40
Age (years)	39.4 (10.5)	46.5 (12.7)
BMI (kg/m ²)	25.3 (4.2)	25.5 (1.8)
Systolic BP (mmHg)	124.7 (10.9)	127.0 (14.6)
Diastolic BP (mmHg)	77.0 (7.9)	80.4 (12.1)
Usual alcohol consumption (U/week)	11.7 (14.4)	11.1 (8.6)
Smoking (% smokers)	5	22
tHcy (µmol/l)	10.0 (8.2–11.6)	10.9 (8.4–13.3)
Folate (ng/ml)	6.0 (4.0–8.6)	4.2 (2.9–5.8)
B ₁₂ (pg/ml)	406 (309–502)	350 (287–444)
Total cholesterol (mmol/l)	5.2 (1.1)	5.2 (1.0)
HDL-cholesterol (mmol/l)	1.1 (0.3)	1.1 (0.2)
Triglycerides (mmol/l)	1.2 (0.8–1.6)	1.4 (1.0–1.8)

Data presented as mean (SD) except for smoking status (presented as % smokers), and tHcy, folate, B₁₂ and triglycerides, where data presented as geometric mean (25th, 75th percentile).

between the MTHFR 677C>T genotypes (data not shown).

Discussion

This study has shown that 2 weeks of moderate alcohol consumption, either as red wine or vodka, increased tHcy and reduced folate and vitamin B₁₂ status.

Previous cross-sectional studies have suggested an association between alcohol consumption and tHcy status,^{19,20,26} but the nature of the relationship has not been fully established. While it is accepted that chronic alcohol intake leads to an increase in tHcy concentrations,^{27,28} not only cross-sectional studies of more moderate drinking habits in the general population have resulted in observed positive associations,^{19,20} but also inverse associations^{26,29} and J-shaped associations.^{30–32} The J-shaped association may help to explain the contrasting results from population-based studies and studies among alcoholics, but this contrast highlights the difficulties of observational studies, and the need for randomized controlled trials to establish fully the effect of alcohol consumption on tHcy.

Observational studies have hypothesized that the association between tHcy and alcohol consumption may depend on type of alcohol consumed.^{10,33,34} Several previous intervention trials have examined

Table 2 Effect of red wine or vodka intervention on tHcy, vitamin B₁₂, folate and lipids in healthy male volunteers

	Analysis of two period crossover trial incorporating adjustment for period									
	vodka intervention					Red wine intervention				
	GM Pre	GM Post	Ratio (95% CI)	P		GM Pre	GM Post	Ratio (95% CI)	P	Vodka vs. red wine
tHcy (μmol/l) n=77	10.4	10.7	1.03 (0.99–1.07)	0.09		10.3	10.7	1.05 (1.01–1.09)	0.03	0.57
Vitamin B ₁₂ (pg/ml) n=78	370	349	0.94 (0.92–0.97)	<0.001		365	344	0.94 (0.92–0.97)	<0.001	0.94
Folate (ng/ml) n=78	4.81	4.36	0.91 (0.86–0.96)	<0.001		4.94	4.63	0.94 (0.89–0.99)	0.03	0.38
Triglycerides (mmol/l) n=79	1.3	1.4	1.11 (1.04–1.07)	0.002		1.3	1.4	1.08 (0.99–1.09)	0.07	0.56
Total cholesterol (mmol/l) n=79	Pre	Post	Difference (95% CI)	P		Pre	Post	Difference (95% CI)	P	
	5.2	5.4	0.21 (0.10–0.31)	<0.001		5.2	5.4	0.13 (0.02–0.24)	0.02	0.35
HDL-cholesterol (mmol/l) n=76	1.13	1.16	0.03 (0.00–0.06)	0.03		1.13	1.15	0.01 (–0.01–0.04)	0.34	0.34

GM: geometric mean; GM ratio: ratio of geometric means (post:pre). Results presented using GM and GM ratio for logarithmically transformed variables and mean and difference for normally distributed variables.

Table 3 Effect of alcohol intervention on tHcy by MTHFR 677C>T genotype in healthy male volunteers

		n	GM ratio	(95%CI)	
Red wine	CC	32	1.030	0.982	1.080
	CT	39	1.030	0.980	1.082
	TT	10	1.034	0.882	1.212
	TT with outlier	11	1.107	0.899	1.364
Vodka	CC	32	1.025	0.974	1.079
	CT	36	0.999	0.951	1.048
	TT	10	1.063	0.980	1.153
	TT with outlier	11	1.132	0.966	1.327

GM ratio: ratio of geometric means (post:pre).

the effect of alcohol consumption on tHcy. The first was a small randomized, diet-controlled crossover study comparing red wine, beer or spirit consumption (Dutch gin) during 3-week intervention periods. It showed that 40 g ethanol/day as red wine and spirits, but not as beer, increased tHcy (red wine by 8% and spirits by 9%) in 11 male volunteers.³⁵ In another study, different levels of alcohol consumption (0, 15, or 30 g—all supplied as 95% ethanol in orange juice) were tested in a crossover design with 8-week intervention periods.³⁶ This study showed that 30 g ethanol/day lowered B₁₂ (by 5%) and raised tHcy (by 3%) in 53 post-menopausal women. There was no effect on folate and no effect of 15 g/day alcohol consumption. In contrast, a parallel group study showed no effect on tHcy of 375 ml red wine daily vs. no alcohol for 2 weeks in non-smoking healthy volunteers.³⁷ Similarly, a comparison of 4 weeks of intervention with either white or red wine (20 g ethanol/d) in 35 healthy women (crossover design) showed no effect of either intervention on tHcy.³⁸ Another randomized crossover trial in men and post-menopausal women (n=19), found that beer consumption (3/4 U a day for women/men for 3 weeks with 1 week washout) did not affect tHcy.³⁹ Finally, a non-controlled study evaluated the administration of 30 ml tequila per day for 30 days in eight healthy non-obese, young male volunteers and showed a 19% increase in tHcy.⁴⁰

The studies described above appear to indicate that beer consumption does not increase tHcy (two studies showing no effect^{35,39}), and that spirits or ethanol do increase tHcy (three studies showing an increase^{35,36,40}). The situation for red wine is less clear, with one study showing an increase in tHcy for red wine,³⁵ and two no effect.^{37,38} Our study adds to this body of evidence, showing

a tHcy-raising effect of both red wine and vodka. The magnitude of the tHcy-raising effect would appear to depend on the number of grams of alcohol consumed, with our raising effect (3–5%, 24 g/d) similar to other studies giving similar amounts of alcohol (3%, 30 g/d),³⁶ whilst studies giving larger amounts of alcohol^{35,40} have shown larger tHcy-raising effects (8–9%,³⁵ 19%⁴⁰).

Only three of these studies looked at B vitamin status.^{35,36,39} Van der Gaag *et al.*³⁵ showed no effect of any alcohol intervention on vitamin B₁₂, a fall in folate with spirits consumption and an increase in vitamin B₆ with all alcohol interventions. In contrast, Laufer *et al.*³⁶ only showed an effect of ethanol on vitamin B₁₂, with no effect on folate. Beulens *et al.*³⁹ showed that beer consumption increased pyridoxal-5-phosphate (the active form of vitamin B₆), seemed to decrease vitamin B₁₂, but had no effect on folate. We showed a significant reduction of both vitamin B₁₂ and folate with red wine and vodka. Laufer *et al.*³⁶ suggested that inadequate vitamin intakes and alcohol consumption may interact to deplete folate and vitamin B₁₂ status, and that, if nutritional intake meets recommended levels, a lowering effect of alcohol on folate in particular may not be observed. We do not have dietary intake data on our participants, and therefore cannot comment on their baseline nutritional status, although folate and vitamin B₁₂ concentrations were in the normal range.

We did not observe a difference in change in tHcy by MTHFR 677C>T genotype. An association has previously been suggested between alcohol, folate, MTHFR 677C>T and tHcy. De la Vega *et al.*⁴¹ showed in hospitalized heavy drinkers what had previously been shown in the general population: tHcy was significantly higher in MTHFR 677TT homozygotes than in MTHFR 677CT heterozygotes and MTHFR 677CC homozygotes, and that this was particularly pronounced in MTHFR 677TT homozygotes with low folate status.¹⁸ Chiuve *et al.*⁴² were able to show in a cross-sectional analysis of 988 women that the association between folate intake and tHcy was modified by both alcohol intake and MTHFR 677C>T genotype. Folate intake was only modestly inversely associated with tHcy among light drinkers (<15 g/d) and non-drinkers, but this association was significantly stronger among moderate drinkers (>15 g/d). In moderate alcohol drinkers, this observed association between tHcy and folate intake was mainly limited to the thermolabile homozygotes (MTHFR 677TT), and although moderate drinkers who were MTHFR 677TT homozygotes had elevated tHcy at low folate intakes, tHcy was no longer elevated with high folate intakes. Therefore, the elevation of tHcy in women who

have low folate intake and are moderate alcohol consumers is magnified in MTHFR 677TT homozygotes, but the effect of alcohol on tHcy in MTHFR 677TT homozygotes would appear to be annulled in those with high folate intake.^{42,43}

This observation may explain why we saw no difference in change in tHcy by MTHFR 677C>T genotype: all our participants had relatively normal folate status. There was a single outlier, a MTHFR 677TT homozygote, whose tHcy increased by >20 µmol/l on both red wine and vodka, but exclusion of this participant did not markedly change the estimates. This outlier had folate concentrations at the lower end of the reference/normal range (2.8 ng/ml vodka baseline; 3.5 ng/ml red wine baseline), and this may be a reason why this participant's tHcy increased so dramatically. It is impossible to formally test for an interaction between folate and the tHcy-raising effect of alcohol in MTHFR 677TT homozygotes with only 11 MTHFR 677TT homozygotes in this dataset. This needs to be tested in studies powered to detect and quantify such an effect.

Conclusion

This study shows that a 2-week alcohol intervention (24 g/day) in healthy male volunteers results in a decrease in folate and vitamin B₁₂ status and an increase in plasma tHcy. The effect of alcohol intervention on tHcy, folate and vitamin B₁₂ concentration did not differ between the red wine and vodka intervention groups. The effect of alcohol consumption on tHcy does not seem to depend on MTHFR 677C>T genotype.

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